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(54) Title: POLYMERS AND PRODUCTS DERIVED THEREFROM

(57) Abstract

Certain fibre-forming and film-forming polyamides, polyurethanes and polyureas have been prepared to have active substituents for covalent attachment of compounds, such as biological compounds having reactive amino or thiol groups. The active substituents are attached to the polymer backbone through an amide, urethane or urea group. These "activated" polymers can be used as affinity separation matrices, cell support media and bioartificial compositions in various shapes and forms.

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POLYMERS AND PRODUCTS DERIVED THEREFROM

Field of the Invention

The invention relates to polymers and to products derived therefrom. More particularly, fibre-forming or film-forming polyamide, polyurethane or polyurea polymers are provided bearing active substituents which permit the covalent attachment of compounds to the polymers. Applications for such "activated" fibres include affinity separation matrices, cell support media and bioartificial composites for biomedical applications.

Background of the Invention

The preparation of polyamide, polyurethane and polyurea polymers is known. For example, polyurethane polymers comprise the reaction products of polyisocyanates and polyhydroxy compounds. Fibres or films can be prepared from solutions or melts of such polymers having a sufficiently high molecular weight.

GB-A-1 530 990 describes the production of electrostatically spun polyurethane tubular products for use as prosthetic structures. Also, GB-A-1 527 592 describes the use of a mat of electrostically spun polyurethane fibres in a product suitable for use as a wound dressing.

There is a need for polymers which can be shaped and whose properties can be modified for a particular application by the covalent attachment of compounds which confer desired properties to the polymer. For example, porous polymeric fibrous structures are required for a variety of applications in addition to those specifically mentioned in the prior art. The surface characteristics required of the fibres would vary depending on the intended use.

Unlike the polyurethane fibres described in the prior art, fibres are needed which possess chemical functionality which renders them susceptible to the covalent attachment of compounds capable of conferring desired properties to the fibre.

Summary of the Invention

The invention provides a fibre-forming or film-forming polyamide, polyurethane or polyurea polymer characterised in that the polymer contains activating groups attached to the polymer through the nitrogen atom of the amide, urethane or urea groups of the polymer, the activating groups being capable of reaction with the amino or thiol group of a compound containing an amino or thiol group to effect covalent attachment of the compound to the polymer e.g. by the formation of an amide or thioether link, respectively.

The invention also extends to the polymer in shaped form e.g. fibrous or film form.

In a further aspect of the invention, the polymer has an amino or thiol group-containing compound covalently attached thereto by the formation of a link by reaction between the activating group of the polymer and the amino or thiol group of the compound.

A method of separating an amino or thiol group-containing compound from a liquid containing the compound comprises passing the liquid through a mat of fibres of the activated polymer of the invention.

A method of separating a receptor compound from a liquid containing the receptor comprises passing the liquid through a mat of fibres of the invention having an amino or thiol group-containing compound covalently attached thereto as

described above wherein the amino or thiol group-containing compound is a ligand for the receptor.

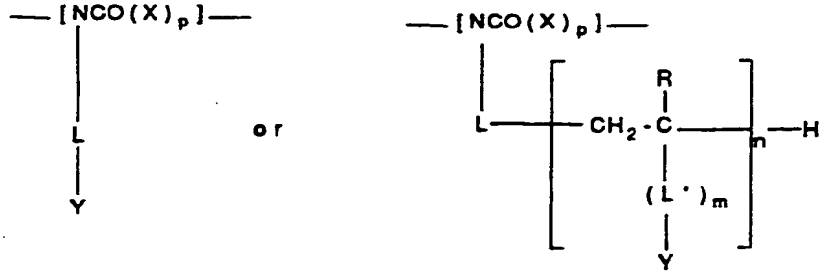
Detailed Description of the Invention

The polymers of the invention can be prepared by modifying any polyamide, polyurethane or polyurea having fibre-forming or film-forming properties. The polymer may be an elastomer and, in a preferred embodiment of the invention, a polyetherurethane is employed. Examples of suitable commercially available polymers from which polymers of the invention can be prepared include, but are not limited to, BIOMER™, PELLETHANE™, TECOFLEX™ and ESTANE™ polymers.

Groups capable of coupling with an amino or thiol group-containing compound e.g. by the formation of an amide or thioether link, respectively, are known.

Preferred activating groups include an imidazolyl carbamate group, a 1-methyl-2-pyrydyl group or a group having the formula -COOZ wherein Z is an electron-withdrawing group. Functional groups are classified as electron-withdrawing groups relative to hydrogen, e.g. $-NO_2$ and -I groups draw electrons to themselves more than a hydrogen atom occupying the same position in the molecule, J. March, Advanced Organic Chemistry, 2nd edition, McGraw Hill, p.20 and 246. Specific examples of Z groups include N-succinimido, benzylidene aniline, pentafluorophenyl, 4-nitrophenyl, 4-cyanophenyl, 4-alkylsulphonylphenyl, acyl, 4-acylphenyl, 4-dialkylaminocarbonylphenyl, 4-alkoxycarbonylphenyl and 4-alkoxysulphonylphenyl.

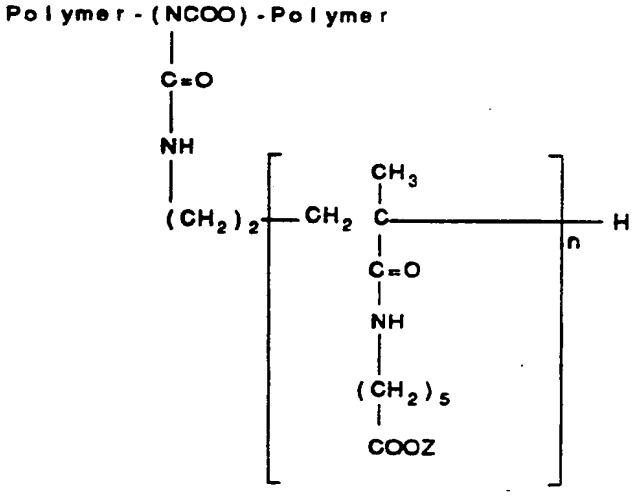
Preferably, the polymer of the invention comprises units having the formula

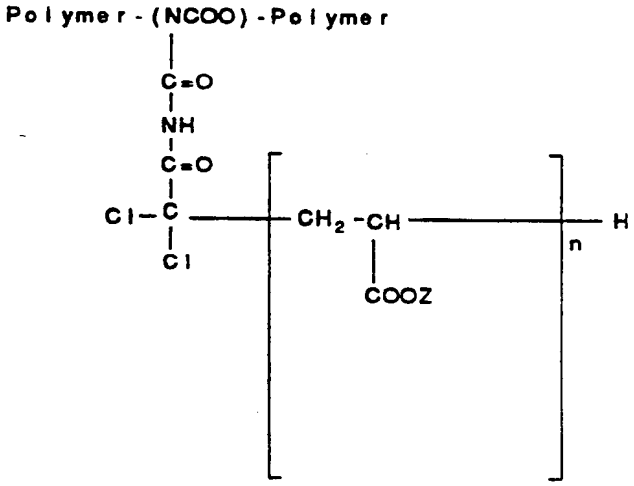


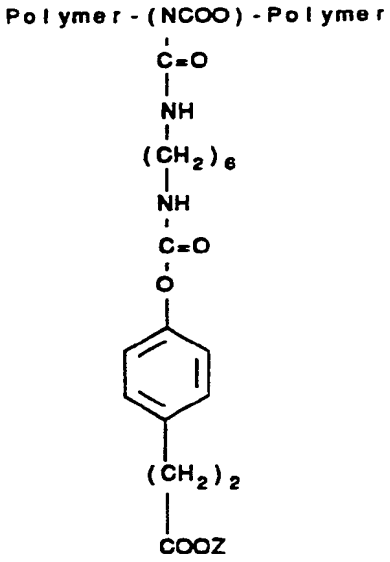
wherein $-[NCO(X)_p]-$ in which X is -O- or -NH- and p is 0 or 1, is an amide, urethane or urea group in the polymer backbone, L and L' are each independently a linking group, R is hydrogen or alkyl, Y is an activating group, m is 0 or 1 and n is an integer from 10 to 150, preferably from 30 to 120.

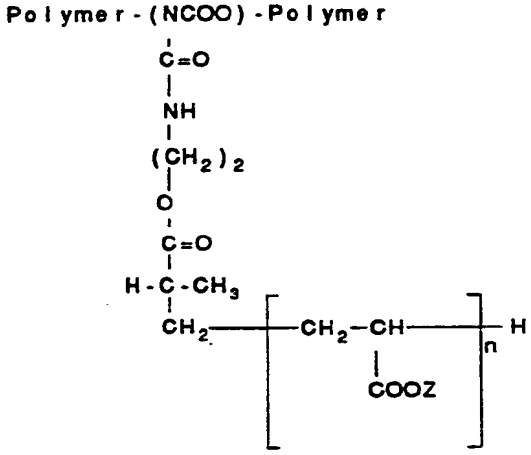
L and L' together with the atoms linking them serve to space the activating group Y away from the polymer backbone. Each of L and L' may comprise one or more divalent hydrocarbon groups such as substituted or unsubstituted alkylene and arylene groups which are connected or terminated with heteroatoms or heteroatom-containing groups such as -O-, -N- , -S-, -NHCO-, -COO- and -CO-. Preferably, L comprises a chain of from 4 to 50 atoms separating the activating group or the activating group-containing moiety from the polymer backbone.

Specific examples of L and L' groups are shown in the following schematic representations of polyurethane polymers of the invention wherein the term "Polymer" is used to indicate the remainder of the polyurethane polymer which contains further urethane groups similarly substituted:

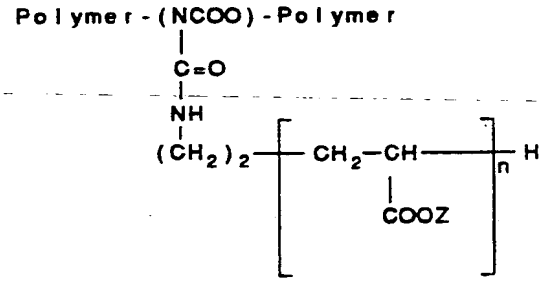

Polymer - (NCOO) - Polymer
C=O
NH
(CH2)2
CH2
CH3
C
H
n
C=O
NH
(CH2)5
COOZ


Polymer - (NCOO) - Polymer
C=O
NH
C=O
Cl
C
Cl
CH2
CH
H
n
COOZ


Polymer - (NCOO) - Polymer
C=O
NH
(CH2)6
NH
C=O
O
(CH2)2
COOZ


Polymer - (NCOO) - Polymer
C=O
NH
(CH2)2
O
C=O
H-C-CH3
CH2
CH2-CH
n
H
COOZ

, and


Polymer - (NCOO) - Polymer
C=O
NH
(CH2)2
CH2-CH
n
H
COOZ

5

In a preferred embodiment of the invention, the amino or thiol group-containing compound is a protein or a polypeptide. For example, the protein may be a ligand suitable for use in affinity chromatography e.g. an antibody. Alternatively, the protein may be a cell-compatible protein such as collagen which could render the polymer suitable for use as a cell support medium. The polypeptide may be a growth factor, e.g. Epidermal Growth Factor (EGF)

The activating group reacts directly with the amino group-containing compound. Preferably, such reaction will take place under physiological reaction conditions.

A number of synthetic methods are available for preparing the polymers of the invention.

One method comprises reacting a fibre-forming or film-forming polyamide, polyurethane or polyurea with a haloisocyanate or an ethylenically unsaturated isocyanate and subsequently grafting an ethylenically unsaturated monomer comprising an activating group onto the product.

Examples of haloisocyanates include haloalkyl and haloacetyl isocyanates e.g. 2-chloroethyl isocyanate and trichloroacetyl isocyanate. Examples of ethylenically unsaturated isocyanates include isocyanato acrylate monomers e.g. isocyanatoethyl methacrylate.

Examples of ethylenically unsaturated monomers comprising an activating group include N-acryloyloxy-succinimide and the succinimide ester of 6-methacrylamidocaproic acid.

Another method of preparing a polymer of the invention comprises reacting a fibre-forming or film-forming polyamide, polyurethane or polyurea with

a diisocyanate and subsequently reacting the product containing free isocyanate groups with a hydroxy-containing reactive ester. Alternatively, the product containing free isocyanate groups may be reacted with an alkanolamine or other amino alcohol, or a diol, to produce a hydroxylated or carboxylated polymer which may subsequently be activated.

Examples of diisocyanates include alkylene and arylene diisocyanates, e.g. hexamethylene diisocyanate and 2,4-tolylene diisocyanate.

Examples of hydroxy-containing reactive esters include hydroxyalkyl, hydroxyaryl, hydroxyalkaryl and hydroxyaralkyl reactive esters, e.g. N-[3-(4-hydroxyphenyl)-propionyloxy]-succinimide.

Examples of compounds used to convert the free isocyanate groups of the polymer to hydroxylated forms include alkanolamines such as ethanolamine, 6-amino-1-hexanol and glucamine, and diols such as poly(ethylene glycol).

Examples of compounds used to convert the free isocyanate groups of the polymer to carboxylated forms include amine group-containing carboxylic acids such as 6-aminocaproic acid.

Examples of components used for activating the hydroxylated polymer are 1,1'-carbonyldiimidazole (CDI) and 2-fluoro-1-methyl pyridinium toluene 4-sulphonate (FMP).

The methods described above may be carried out in solution such that the polymer is dissolved prior to reaction. In this way, an activated polymer is formed which may subsequently be shaped into the desired fibrous or film form.

Alternatively, the polymer in solid form e.g. in fibrous or film form may be treated with

solutions of the reactants so that only the surface of the polymer is activated.

In a preferred embodiment of the invention, the activated polymer is provided in fibrous form. The fibres may be produced by electrostatic spinning in accordance with the teaching of GB-A-1 530 990. The activated polymer of the invention may be spun into fibres. Alternatively, a polymer may be spun into fibres and then modified by the attachment of activating groups.

In the electrostatic spinning process, the fibres are collected as a porous mat on a suitably located receiver. In this way, a substrate coated with a layer of the fibres can be produced. Alternatively, the fibrous mat can be stripped from the receiver.

The fibrous product can be produced in a variety of shapes. For example, by using a cylindrical receiver, a tubular product can be made.

The fibres obtained by the electrostatic spinning process are thin and can be of the order of 0.1 to 25 μm in diameter. Fibre diameters of 0.5 to 10μm, especially 1.0 to 5 μm may be preferred.

The polymer may be conveniently spun from solution. Suitable solvents include dimethylformamide, N,N-dimethylacetamide, dichloromethane and methyl ethyl ketone. Solvent mixtures may be preferred, such as a mixture of N,N-dimethylformamide and methyl ethyl ketone (1.45:1 weight ratio). The concentration of the polymer in solution will depend upon the amount required to provide adequate fibre properties and will be influenced by the need to produce a liquid of appropriate viscosity and speed of fibre hardening.

For example, a preferred concentration when using BIOMER^{TM} polymer, a commercially available poly(etherurethaneurea) having a molecular weight in the region of 60,000, dissolved in N,N-dimethylacetamide is from 10 to 20% w/w, for example, 16% w/w.

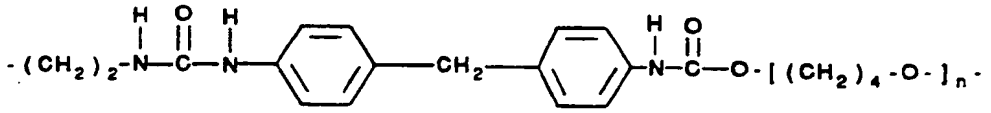
Any convenient method may be employed to bring the polymer solution into contact with the electrostatic field for spinning. For example, the solution may be supplied to an appropriate position in the electrostatic field by feeding it to a nozzle from which it is drawn by the field to form fibres. The solution may be fed from a syringe reservoir to the tip of a grounded syringe needle, the tip being located at an appropriate distance from an electrostatically charged surface. Upon leaving the needle, the solution forms fibre between the needle tip and the charged surface. The electrostatic potential employed may be conveniently from 10 to 100 Kv, preferably from 10 to 50 Kv.

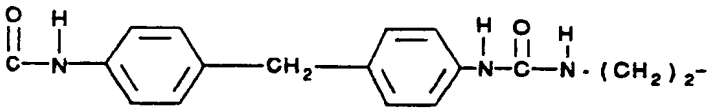
The pore size and porosity of the fibrous product may be controlled, for example, by varying such parameters as the diameter of the fibres and their density of deposition.

Typically, the fibrous product comprises a network of very fine fibres having a diameter of approximately 1 µm. The fibres are melded at many junction points and enclose irregular holes or pores with a typical dimension in the range from 5 to 10 µm. The overall surface area of the fibres is extremely large. For example, 1 g of the fibrous material may have a total surface area of approximately 4 m^2.

The invention is further illustrated by way of example as follows. In the following Examples,

-(NHCOO)- represents a urethane group in the polyurethane polymer used. BIOMER™ polymer is a commercially available poly(etherurethaneurea) having the structure:





The molecular weight (MnW) of the polymer is about 60,000.

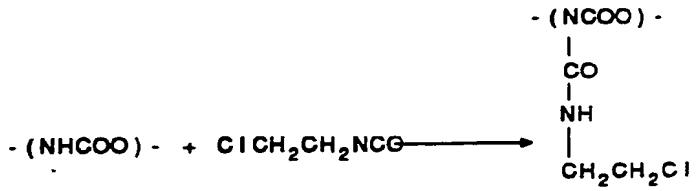
Example 1

The synthesis of a pre-activated polyetherurethane was effected in the three steps described below.

1. Functionalisation of polyetherurethane.

A polyetherurethane (BIOMER™, Ethicon, Someville, NJ: 30 g) was dissolved in N,N-dimethylacetamide (DMAC) (50 ml). 2-Chloroethyl isocyanate (4 ml) was added to the resultant solution. The reaction mixture was kept for 3 days at room temperature and then precipitated into water. After precipitation the polymer was filtered off, washed carefully with water and then dried in a vacuum oven.

The reaction of the polyetherurethane with 2-chloroethyl isocyanate gives an allophanate product (I), as represented by the following equation:



Chlorine analysis of the functionalised polyetherurethane (I) produced: % Cl = 1.12

2. Synthesis of the spaced active ester monomer, the N-succinimido ester of 6-methacrylamidocaproic acid (II).

6-Aminocaproic acid (26.2 g, 0.2 moles) was dissolved in a solution of sodium hydroxide (8.0 g) in water (25 ml). TOPANOL OC™, a commercially available surfactant from ICI comprising 4-methyl-2,6-tertiary-butyl phenol, was added, and the solution cooled to -10°C. A solution of methacryloyl chloride (20.8 g, 0.2 moles) in dioxane (15 ml) was then added simultaneously with a solution of sodium hydroxide (8.0 g) in water (20 ml) over a period of 1 hour. The latter two solutions had been cooled in an ice-bath prior to their addition. On completion of the addition, the reaction was stirred for a further 2 hours at -10°C. The reaction mixture was then left to stand overnight in the refrigerator.

After standing overnight, the reaction mixture was adjusted to pH 4 with dilute hydrochloric acid. The solution was concentrated using a rotary evaporator, and the residue extracted with ethyl acetate. The combined extracts were washed with water and dried over magnesium sulphate. The solution was filtered and the solvent removed at the rotary evaporator.

Ethyl acetate/petroleum ether (60-80°C) was added to the (oil) residue. This resulted in separation into an oily layer and a cloudy solvent

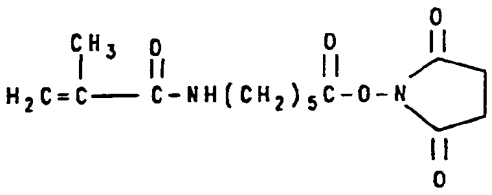
layer. The solution was shaken vigorously and, upon settling, the cloudy solvent layer was removed and retained. Further ethyl acetate/petroleum ether (60-80°) was added and the above successively repeated until the oily layer was no longer observed. The product was obtained by cooling the combined extracts in an ice-salt bath and scratching (yield: 22.6 g, 70%).

6-Methacrylamidocaproic acid (10 g, 0.05 moles) and N-hydroxysuccinimide (5.75 g, 0.05 moles) were placed in a three-necked flask that was fitted with a magnetic stirrer, air condenser (with calcium chloride guard tube) and a dropping funnel. Dichloromethane (50 ml) and tetrahydrofuran (10 ml) and 4-methylaminopyridine (4-DMAP) (0.12 g) were added, and the solution was stirred in an ice bath. A solution of dicyclohexylcarbodiimide (DCCI) (11.5 g) in dichloromethane (20 ml) was added dropwise. The urea precipitated in due course and the reaction was allowed to run overnight.

The solid (urea) precipitate was filtered off and washed with dichloromethane. The combined washings and filtrate were stripped on the rotary evaporator. The residual oil was dissolved in acetonitrile and the solution was cooled in the refrigerator for 2 hours. The small amount of urea which had precipitated was filtered off and the solvent was then removed under vacuum. The remaining oil was dissolved in ethyl acetate. The solid product precipitated on standing in an ice-salt bath, and was filtered and dried.

Analytical and spectroscopic data were consistent with the required structure (II).

Yield: 4.29 g (29%); mp 77.5 °C.

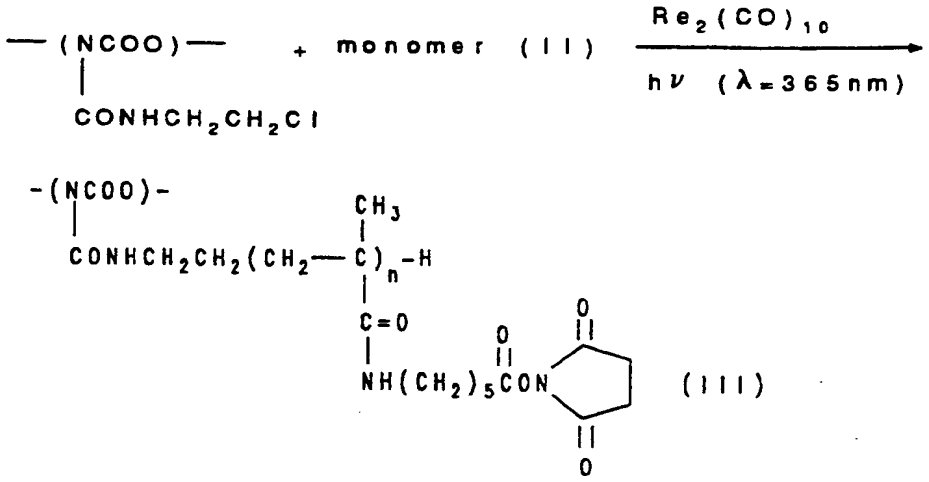


(II)

3. Grafting monomer (II) to functionalised polyetherurethane.(I)

The functionalised polyetherurethane (I) (7.56 g) was dissolved in pure DMAC (60 ml). A solution of 1.56 g of monomer (II) in DMAC (5 ml) was then added to this, together with a solution of 0.053 g of $Re_2(CO)_{10}$ in DMAC (2 ml). The reaction mixture was then degassed under vacuum and sealed off. The grafting was carried out photochemically (λ = 365 nm) for 7 hours at room temperature in accordance with the teaching of C.H. Bamford in Reactivity Mechanism & Structure in Polymer Chemistry ed. A.D. Jenkins & A. Ledwith, John Wiley 1974, Chapter 3. The polymer solution was then precipitated into a mixture of a very dry diethyl ether/ethyl acetate (9:1). After precipitation, the polymer product was filtered off and washed with diethyl ether, vacuum dried and weighed. The weight increase - corresponding to grafting of the polymer of (II) onto (I) to generate the pre-activated polyetherurethane (III) - was 8.14%.

The photochemically initiated grafting reaction is represented in the following equation.



Using the procedure described above, the functionalized polymer (I) was also reacted with N-acryloxy succinimide to provide an activated polymer of this invention.

Example 2

The pre-activated polymer (III) was dissolved in bulk DMAC to obtain a concentration suitable for electrostatic spinning (16% w/w). The solution was spun at minimum humidity following the procedure given in GB-A-1 530 990 to produce the required sheet of fibrous pre-activated polyetherurethane.

The sheet was cut into strips measuring 2 x 1 cm. Samples of the strips were immersed in a solution of radiolabelled Protein A (1.0 ml, 1 mg Protein A/ml 0.1 molar sodium hydrogen carbonate buffer, pH 8). The strips were left to stand for 2 hours at room temperature. The strips were then removed, washed first in excess buffer and then in deionised water before blotting dry on a filter paper.

The strips were allowed to stand in a solution of sodium dodecyl sulphate (SDS) (5 ml, 2% by

weight) for one hour at room temperature. They were then washed with deionised water and dried.

Each strip was then counted for one minute in a scintillation counter and compared with a reference to determine the quantity of Protein A covalently bound to the polyetherurethane.

The results showed that Protein A was coupled successfully to the polymer at a level of about 34 mg/m^2.

The above procedure was repeated using radiolabelled human IgG (Sigma Chemical, 1 mg/ml) instead of Protein A. The results showed that the human IgG was coupled successfully to the polymer also at a level of about 34 mg/m^2.

The binding activity of the Protein A coupled to the polymer was assessed as follows.

Four strips of the pre-activated polyetherurethane (2 x 1 cm each) were added to a solution of Protein A (2.5 ml, 1.0 mg/ml) in coupling buffer, 0.1 molar sodium hydrogen carbonate, pH 8. The strips were incubated for two hours at room temperature, washed with coupling buffer followed by water and blotted dry on filter paper.

The strips were then added to a blocking reagent (1 molar ethanolamine pH 8, 5 ml) and left to stand for one hour at room temperature. The strips were washed with water and stored in 0.15 molar PBS at approximately 4°C.

The strips of polyetherurethane having Protein A coupled thereto were placed in a solution of radiolabelled human IgG (1.0 mg/ml, 1 ml) for one hour at room temperature. The strips were removed, washed with water and placed in 0.15 molar PBS containing 0.2% TWEEN™ 20 nonionic surfactant (5 ml) for five minutes to remove non-specifically bound protein. The

strips were rewashed with water and blotted dry on filter paper. Each strip was counted for one minute using a scintillation counter to provide a measure of specific binding.

Other strips of (unlabeled) polyetherurethane having Protein A coupled thereto were incubated for one hour in a solution of radiolabelled Protein A made up in 0.15M PBS/TWEEN™ 20 nonionic surfactant as above to provide a measure of non-specific binding of protein.

Reference strips were prepared by adsorbing a known quantity of radiolabelled human IgG (1 mg/ml) on the polyetherurethane and counted.

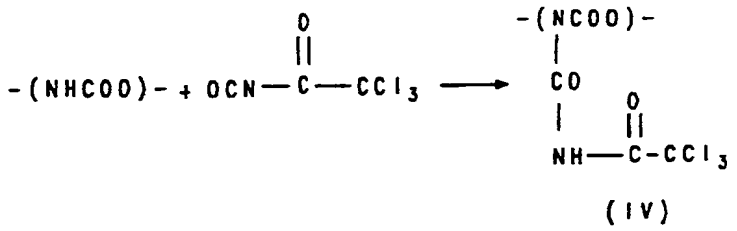
The results showed that the specific binding of human IgG to the polyetherurethane having Protein A coupled thereto was about 92 mg/m^2. The non-specific binding of protein to the equivalent sample was found to be about 18 mg/m^2.

Example 3

An electrostatically spun polyetherurethane (BIOMER™) tube suitable for use in arterial prosthesis was modified as follows.

1.Functionalisation of the polyetherurethane.

The fibrous tube was reacted with trichloroacetyl isocyanate (3 g, 0.016 mole) in 150ml hexane for 24 hours. After this time the tube was washed off with water very carefully and subsequently immersed in water for 2 days and vacuum dried. The tube showed a positive chlorine test. The overall reaction of the polyetherurethane and trichloroacetyl isocyanate is depicted in the following equation:

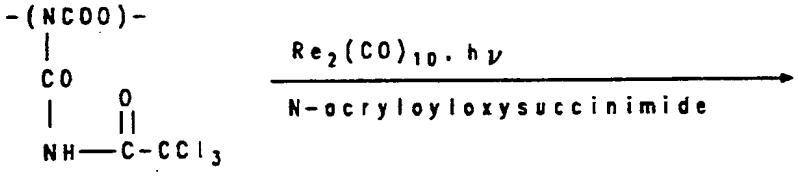


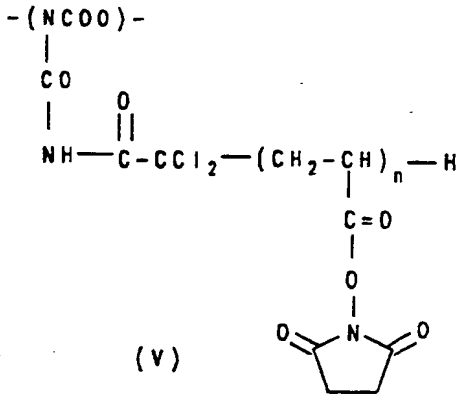
(IV)

2.Grafting of N-acryloyloxysuccinimide

The fibrous tube of functionalized polyetherurethane (IV) was placed in a reaction vessel and a solution of $Re_2(CO)_{10}$ (0.095 g 0.00014 mole) and N-acryloyloxysuccinimide (0.75 g, 0.0044 mole) in 25 ml dry ethyl acetate was added. The reaction mixture was degassed under vacuum and the vessel sealed off. The reaction solution was photolysed at λ = 365nm at ambient temperature for 2 hours. Then the irradiation was continued under a 60 watt lamp for 24 hours with continuous rotation. The tube was then washed thoroughly with dry ethyl acetate and vacuum dried.

The chemical structure of the grafted polyetherurethane (V) is shown as follows:





(V)

3.Covalent attachment of collagen.

The grafted tube of polyetherurethane (V) was reacted with 1% w/v suspension collagen (type I) in 0.05 molar acetic acid for 2 hours. The tube was dried at room temperature overnight and then in a vacuum for 24 hours. The tube was washed thoroughly in distilled water and vacuum dried. Scanning electron micrographs revealed that the whole fibrous structure was covered with a layer of collagen

Example 4

Polyetherurethane (BIOMER™) was dissolved in DMAC to form an 8% w/w solution. The solution was cast on a glass surface to produce a film. After evaporation of the solvent, the film was immersed in water and stripped from the glass surface. The film was washed extensively with water and dried.

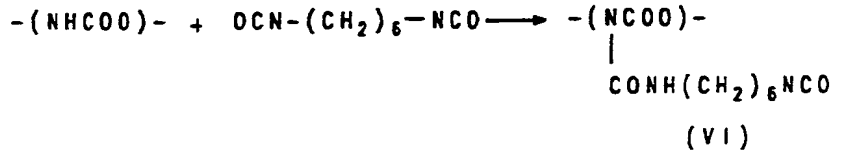
The film of polyetherurethane was treated in a manner identical to that described for the fibrous tube in steps 1 to 3 of Example 3.

Scanning electron micrographs revealed that the surface of the film was completely covered with a layer of collagen.

Example 5

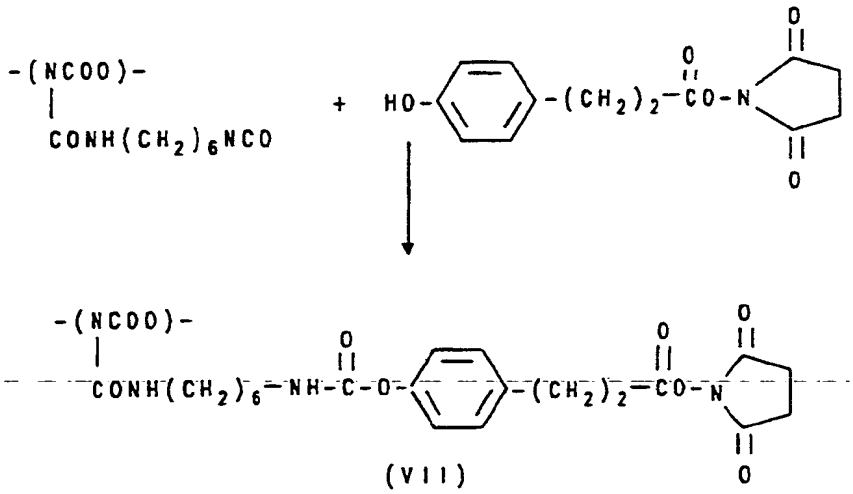
A sample of polyetherurethane (BIOMER™) was activated after electrostatic spinning by the method described below.

A sheet of the electrostatically spun polymer was immersed in hexane for one hour prior to addition of 10 g (an excess) of hexamethylene diisocyanate and the reaction was left standing at room temperature for 4 days. After this time the sheet was removed and carefully washed with hexane and vacuum dried. The reaction is illustrated as follows:



(VI)

The sheet of polymer (VI) was placed in a flask containing a solution of 0.4 g of N-[3-(4-hydroxyphenyl)propionyloyl]succinimide (Fluka) in 40 ml of dry acetonitrile. The flask was wrapped in foil and stirred at room temperature for 5 days. After this time the sheet was removed and carefully washed with an excess of acetonitrile and vacuum dried. The activated polymer was produced according to the following equation.



(VII)

The above procedure was also carried out using 2,4-tolylene diisocyanate instead of

hexamethylene diisocyanate to provide an activated polymer of this invention.

A solution of radiolabelled Protein A was prepared containing 1 mg Protein A/ml 0.1 molar sodium hydrogen carbonate, pH 8. A sample (2 ml) of the resulting solution was passed through a Millipore filter containing a disc (diameter = 2.54 cm) of the polymer (VII) at a flow rate of 1 ml/hour using a syringe-pump. After two hours the disc was removed and washed with 0.1 molar sodium hydrogen carbonate and deionised water. The disc was left standing for one hour in 10 ml sodium dodecyl sulphate (SDS) (2%), washed with deionised water and blotted dry. The disc was then counted for 1 minute in a vial using a scintillation counter. The results of the counting (CPM), the amount of protein A covalently bound to the polymer and also the amount of Protein A physically adsorbed on the unactivated polymer (BIOMER™) as a control are shown in Table 1 below.

Table 1

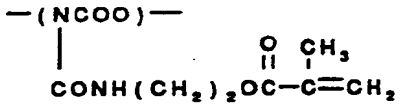
Sample	Sample Wt. (g)	CPM	Protein A mg/g	Protein A mg/m^2
Protein A (I-125)	0.0001	288,382		
Polymer (VII)	0.1125	1,029,354	3.1728	704.42
BIOMER™	0.1086	11,600	0.0370	7.93

Example 6

A sample of polyetherurethane (BIOMER™) was activated after electrostatic spinning by the method described below.

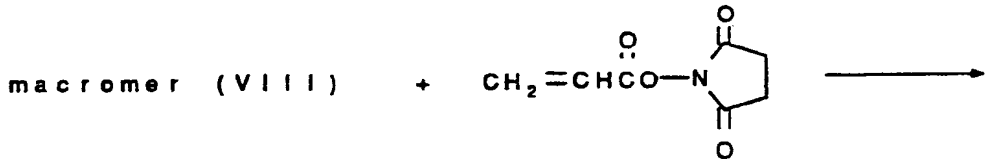
The electrostatically spun polymer was reacted with isocyanatoethyl methacrylate monomer (20% v/v in hexane) at room temperature for 5 days. After this time the functionalized polyetherurethane was

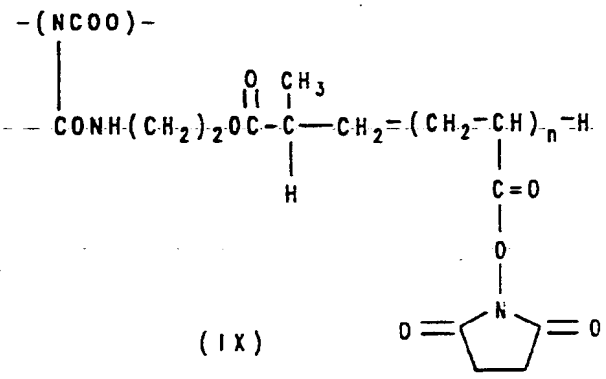
washed with hexane, methanol, water and methanol, respectively. The reaction is shown as follows.



(VIII)

A specimen of macromer (VIII) (1.9 g) was placed in a reaction vessel containing a mixture of 0.5 g N-acryloyloxysuccinimide in 10 ml of dry acetonitrile and 0.2 g azobisisobutyronitrile (AIBN) dissolved in 10 ml acetonitrile. After degassing, the polymerization was carried out at 60°C for 4 hours. The macromer sheet was removed and washed with acetonitrile and vacuum dried. The resulting product (IX) was produced in accordance with the following reaction.





(IX)

Protein A was bound to a sample of polymer (IX) following the procedure given in Example 5. The results are shown below in Table 2.

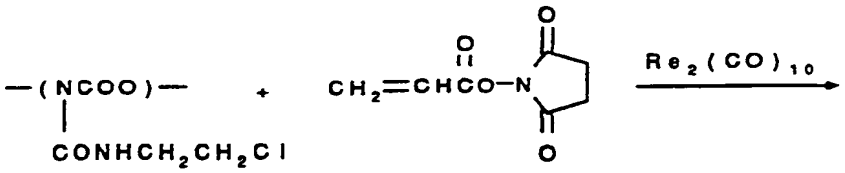
Table 2

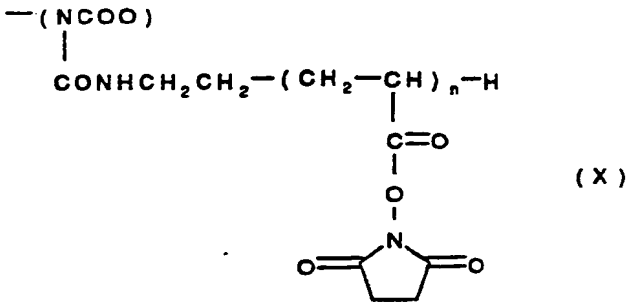
Sample	Sample Wt. (g)	CPM	Protein A mg/g	Protein A mg/m^2
Protein A (I-125)	0.0001	288,382		
Polymer (IX)	0.1103	192,849	0.6062	132.00
BIOMER™	0.1086	11,600	0.0370	7.93

Example 7

A sample of polyetherurethane (BIOMER™) was activated after electrostatic spinning by the method described below.

The electrostatically spun polyetherurethane sheet was reacted with 2-chloroethyl isocyanate (1 g in 20 ml of hexane) for 24 hours at room temperature. After this time, the sheet was washed with hexane, methanol, water and methanol, respectively, and vacuum dried. A graft copolymer (X) was synthesized by grafting N-acryloyloxysuccinimide monomer (0.5 g in 10 ml acetonitrile) on to the chloroethyl isocyanated polyetherurethane in the presence of $Re_2(C0)_{10}$. The reaction is shown as follows.





(X)

Protein A was bound to a sample of polymer (X) following the procedure given in Example 5. The results are shown below in Table 3.

Table 3

Sample	Sample Wt. (g)	CPM	Protein A mg/g	Protein A mg/m^2
Protein A (I-125)	0.0001	288,382		
Polymer (X)	0.0843	403,335	1.6590	276.00
BIOMER™	0.1086	11,600	0.0370	7.93

Examples 8 to 18

Synthesis

Two samples of electrostatically spun BIOMER™ polymer sheet (2 g each) were placed in two reaction vessels. The first one was reacted with 30% hexamethylene diisocyanate in petroleum ether (b.p. 60-80°C) at 40°C, and the second reacted with bulk tolylene 2,4-diisocyanate at room temperature. The reaction time for these samples was five days, after which time the two samples were washed carefully with petroleum ether and vacuum dried.

Each of the isocyanated BIOMER™ polymer samples was reacted with bulk ethanolamine (25 ml) for 17 hours at room temperature to produce hydroxylated polymers.

One sample (1 g) of the hydroxylated BIOMER™ which had been isocyanated with hexamethylene diisocyanate was reacted with 0.5 g (1.7 mmole) of FMP dissolved in 10 ml of dry acetonitrile in the presence of triethylamine (0.2 ml) to give an activated polymer of the invention (Example 8). The reaction was carried out at room temperature for 24 hours. After this time, the sample was washed with dry acetonitrile and dried in a vacuum.

Another sample (1 g) of the same hydroxylated BIOMER™ polymer was reacted with CDI (0.5 g, 3 mmole) dissolved in acetonitrile to give an activated polymer of the invention (Example 9). The reaction was carried out at room temperature for 24 hours. After this time the sample was washed with dry acetonitrile and dried in a vacuum.

The same procedures as above were repeated for the activation of two samples of the hydroxylated BIOMER™ polymer which had been isocyanated with tolylene 2,4 diisocyanate. The sample activated with FMP gave an activated polymer of the invention (Example 10). Similarly, the sample activated with CDI gave an activated polymer of the invention (Example 11).

Following the procedures given above, other polyurethane samples isocyanated with hexamethylene diisocyanate and 2,4-tolylene diisocyanate, respectively, were converted to hydroxylic forms and activated with CDI. More particularly, polymer samples isocyanated with hexamethylene diisocyanate were converted to

hydroxylic forms by reaction with 6-amino-1-hexanol (Example 12) and glucamine (Example 15). Polymer samples isocyanated with 2,4-tolylene diisocyanate were converted to hydroxylic forms by reaction with 6-amino-1-hexanol (Example 13), poly(ethylene glycol)(Molecular weight 4000) (Example 14) and glucamine (Example 16).

Carboxylated polymers were prepared from polyurethane samples isocyanated with hexamethylene diisocyanate and 2,4-tolylene diisocyanate, respectively. The isocyanated polymer samples were reacted with 6-aminocaproic acid and the resulting carboxylated polymers were activated using CDI (Examples 17 and 18, respectively).

Coupling of Protein A to activated BIOMER™ polymer

A disc of each of the polymers of Examples 8 to 18 (diameter = 2.54 cm) was tested with Protein A solution. Each disc was placed in a Millipore filter holder and 3 ml of Protein A labeled with ^{125}I (1 mg/ml solution, prepared from Protein A ^{125}I (Amersham, 10 mCi) diluted with 20 mg of Protein A (Sigma) in 0.1 molar sodium hydrogen carbonate, pH 8) was passed through the disc at a flow rate of 1 ml/hour using a syringe pump. After three hours, each sample was washed extensively with 0.1 molar sodium hydrogen carbonate, followed by deionised water and then left standing in 10 ml of SDS (2%) for one hour. Each sample was then washed with deionised water, blotted dry and counted for one minute in a vial containing 8 ml of Optiphase scintillant. The result of counting (in cpm) and the amount of Protein A covalently bound to the activated supports together with a control are shown in Table 4 below.

Table 4

Sample	Weight (g)	Activity (cpm)	Protein A(mg/g of BIOMER™)
Protein A	0.0002	33399	--
Example 8	0.13	143412	0.66
Example 9	0.0755	789072	6.26
Example 10	0.1043	51723	0.29
Example 11	0.1093	114798	0.63
Example 12	0.320	------	1.81
Example 13	0.306	------	1.46
Example 14	0.3032	------	1.43
Example 15	0.3159	------	2.94
Example 16	0.3494	------	3.04
Example 17	0.3083	------	3.64
Example 18	0.3280	------	4.16
Control	0.0918	2185	0.0143

Example 19

Protein A Coupling

Three discs (diameter = 2.54 cm) of polymer VII above and the polymer of Example 9 were each placed in a Millipore filter holder and 5 ml of protein A (1 mg/ml solution) in 0.1 molar sodium hydrogen carbonate (pH 8) passed through at a flow rate of 1 ml/hour using a syringe pump. After 5 hours, the samples were washed with 0.1 molar sodium hydrogen carbonate followed by water and blotted dry on filter paper. The samples were then reacted with blocking reagent (ethanolamine; pH 8; 10 ml) and left to stand for one hour at room temperature. The samples were washed with water and stored in PBS at 4°C. A similar procedure was carried out using unreacted BIOMER™ polymer as a control.

IgG Binding

Three discs of each sample of protein A/polymer VII and protein A/polymer of Example 9 were

placed in a Millipore filter holder and a solution of radiolabeled (labeled with ^{125}I) human IgG (2.8 mg/ml, 5 ml) in 0.15 molar PBS (pH 7) was passed through at a flow rate of 1 ml/hour. After 5 hours, the discs were removed, washed with water and placed in 0.15 molar PBS containing 0.2% TWEEN™ 20 nonionic surfactant for one hour to remove the non-specifically bound protein. The discs were washed with water and blotted dry on filter paper. Each disc was counted for one minute in 8 ml Optiphase scintillant. The results of counting (in cpm) and the amount of IgG bound to the protein A/polymer supports together with the results for the control are shown in Table 5 below.

Table 5

Sample	Weight (g)	Activity (cpm)	IgG (mg/g polymer)
IgG (labeled)	0.0005	243948	--
Polymer (VII)	0.2876	338260	2.68
IgG (labeled)	0.0003	140527	--
Polymer (Ex. 9)	0.3713	744860	3.96
Control	0.3619	8613	0.02

IgG Coupling

IgG was coupled to samples of polymer VII and the polymer of Example 9 directly. In this case three discs of each of these samples were placed in two Millipore filter holders and labeled IgG (^{125}I) (2 mg/ml, 5 ml) in PBS solution passed through at a flow rate of 1 ml/hour. After this time, the discs were removed, washed with water and placed in 10 ml of SDS (2%) for one hour. Each disc was then washed with

water and counted for one minute in a vial containing 8 ml Optiphase scintillant. The results of IgG bound to the supports are shown in Table 6 below.

Table 6

Sample	Weight (g)	Activity (cpm)	IgG (mg/g polymer)
IgG (labeled)	0.0005	165237	--
Polymer VII	0.2390	401526	5.65
Polymer (Ex. 9)	0.1834	310775	5.70
Control	0.2527	1456	0.019

Example 20

A solution of Epidermal Growth Factor (EGF) labeled with ^{125}I in 0.1 molar sodium bicarbonate (50 µg/3 ml) was passed through a disc (d = 2.54 cm) of electrostatically spun BIOMER™ polymer post-activated with N-[3-(4-hydroxyphenyl)propionyloyl]succinimide (VII) placed in a Millipore filter.

Similarly, a disc of unmodified Biomer was placed in another Millipore filter and used as a control.

The solution of EGF was passed through the two filters with the aid of a multi-syringe pump at a flow rate of 1.5 ml/hr. After both solutions were passed through, the discs were washed with an excess of 0.1 molar sodium bicarbonate, phosphate buffer solution (PBS) and deionised water, respectively.

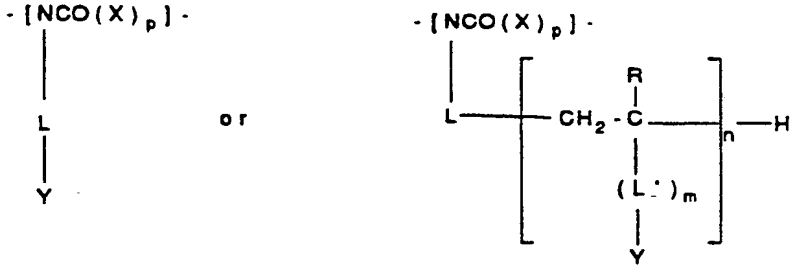
Each disc was immersed in 5 ml of 2% SDS for one hour and washed with deionised water and blotted dry. The amount of EGF coupled to the post-activated BIOMER™ (VII) polymer was estimated as 4 µg/disc. No significant radioactivity was detected on the control sample.

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention. All patents, patent applications (published or unpublished, domestic or foreign), scientific literature, books and other prior art cited herein are all incorporated herein by reference for the teaching therein pertinent to this invention.

CLAIMS:

1. A fibre-forming or film-forming polyamide, polyurethane or polyurea polymer comprising activating groups attached to said polymer through the nitrogen atom of the amide, urethane or urea groups of said polymer, the activating groups being capable of reaction with an amino or thiol group of a compound containing an amino or thiol group to effect covalent attachment of the compound to the polymer.

2. The polymer of claim 1 comprising units having the formula



wherein $-[NCO(X)_p]-$ in which X is -O- or -NH- and p is 0 or 1, is an amide, urethane or urea group in said polymer backbone, L and L' are each independently a linking group, R is hydrogen or alkyl, Y is an activating group, m is 0 or 1 and n is an integer from 10 to 150.

3. The polymer of claim 1 or claim 2 wherein said activating group is selected from the group consisting of an imidazolyl carbamate group, a 1-methyl-2-pyrydyl group and a group having the formula -COOZ wherein Z is an electron-withdrawing group.

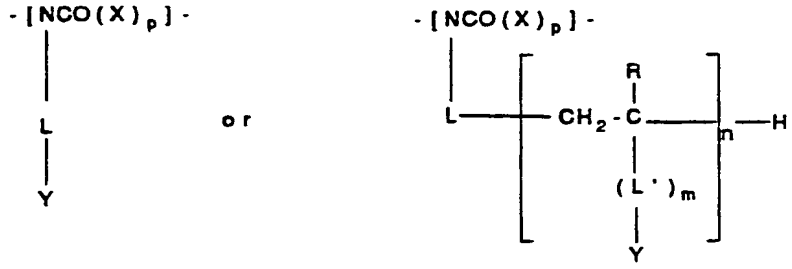
4. The polymer of claim 2 wherein L comprises one or more substituted or unsubstituted alkylene or arylene groups which are connected or

terminated with heteroatoms or heteroatom-containing groups, and L provides a chain of from 4 to 50 atoms which separates the activating group or the activating group-containing moiety from said polymer backbone.

5. The polymer of claim 1 wherein said polymer is a polyetherurethane.

6. A mat of polymer fibres comprising a polyamide, polyurethane or polyurea polymer comprising activating groups attached to said polymer through the nitrogen atom of the amide, urethane or urea groups of said polymer, the activating groups being capable of reaction with an amino or thiol group of a compound containing an amino or thiol group to effect covalent attachment of the compound to the polymer.

7. The mat of claim 6 wherein said polymer comprises units having the formula



wherein $-[NCO(X)_p]-$ in which X is -O- or -NH- and p is 0 or 1, is an amide, urethane or urea group in said polymer backbone, L and L' are each independently a linking group, R is hydrogen or alkyl, Y is an activating group, m is 0 or 1 and n is an integer from 10 to 150.

8. The mat of claim 6 wherein said polymer has an amino or thiol group-containing compound covalently attached thereto by the formation

of a link by reaction between the activating group of the polymer and the amino or thiol group of the compound.

9. The mat of claim 8 wherein said amino or thiol group-containing compound is a protein or a polypeptide.

10. A method of separating an amino or thiol group-containing compound from a liquid containing said compound comprising passing the liquid through a mat of fibres according to claim 6.

11. A method of separating a receptor compound from a liquid containing said compound comprising passing the liquid through a mat of fibres according to claim 8 wherein the amino or thiol group-containing compound is a ligand for said receptor.

12. The polymer of claim 1 having an amino or thiol group-containing compound covalently attached thereto by the formation of a link by reaction between the activating group of the polymer and the amino or thiol group of the compound.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/00129

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C08G18/83; C08G18/10; C08G18/71; C08G69/48
A61L27/00; A61L15/44; C07K17/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C08G ; A61L ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	DE,A,3 523 615 (CYTOMED) 15 January 1987 see claims 1,4-7 see column 2, line 47 - line 67	1,2,12
X	US,A,4 880 883 (T.G.GRASEL ET AL.) 14 November 1989 see claims 1,11 see column 7, line 18 - line 38 see column 8, line 5 - line 46	1,12
X	EP,A,0 367 489 (BAXTER INTERNATIONAL) 9 May 1990 see claims 1,6,11,18 see page 3, line 43 - line 50 see page 5, line 24 - line 42	1,2,12
X	WO,A,8 602 654 (MEMTEC LTD.) 9 May 1986 see claims 1-19 see page 4, line 14 - line 22	1,12

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 MARCH 1992	09.04.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	VAN PUYMBROECK M. A.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	DE,A,2 427 948 (RAMOT UNIVERSITY) 9 January 1975 see claims 1-8 see page 2, paragraph 4 see page 3, paragraph 1 - page 4, paragraph 2 see examples 6,8	1,6,12
A	FR,A,2 240 233 (NATIONAL RESEARCH) 7 March 1975 see claims 1-11 see page 2, line 16 - page 3, line 10 see page 4, line 7 - line 21	1
A	BIOMATERIALS vol. 9, no. 1, January 1988, GUILDFORD,SURREY,GB pages 36 - 46; W.G.PITT ET AL.: 'ALBUMIN ADSORPTION ON ALKYL CHAIN DERIVATIZED POLYURETHANES' see page 45, left column, paragraph 3 - right column, paragraph 3	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9200129 SA 55232

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 24/03/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-3523615	15-01-87	EP-A,B 0214392	18-03-87
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		DE-A- 2437870	20-02-75
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		NL-A- 7410564	10-02-75
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		SE-A- 7409940	07-02-75

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82